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NON-ELECTROMAGNETIC FACTORS INFLUENCE BEHAVIORAL EFFECTS OF MICROWAVE EXPOSURES

by

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16. ABSTRACT Alteration in animal behavior both during and after microwave irradiation is well documented. This report reviews a number of experiments in which specific behavioral effects in laboratory rodents were quantitatively changed by aspects of the exposure such as ambient air temperature, duration of the exposure, and orientation in the field while exposure parameters of 2450 MHz (CW) electromagnetic radiation were kept constant. Operant response rates were reduced and response durations were increased after overnight irradiations with 10 or 15 mW/cm^2 when ambient air temperature during the exposure was 28°C but not when the temperature was 22°C. Operant behavior of rats which was reduced by 50% or more after an overnight irradiation was not reduced after a similar exposure of only 55 min. In another experiment, response rates declined with time after exposures lasting 4 hrs., did not decline further after exposures lasting from 4 to 8 hrs., but did decline further after exposures lasting longer than 8 hrs. Rats did not change orientation or movement in relation to the electric or magnetic field vectors during irradiation with 15 mW/cm^2 whereas mice showed increased movement but no preferred change in orientation during exposures with a similar incident power level. During these exposures field orientation would not alter the absorbed energy (specific absorption rate, SAR) in the rat but would in the mouse because of the relationship of body size to wavelength of the radiation.

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It is well established that animal behavior can be altered both during and after irradiation with microwaves. A number of experiments are reviewed in which specific behavioral effects were quantitatively altered by varying aspects of the exposure other than the parameters of electromagnetic radiation. Operant behavior of rats was assessed by measuring the rate of food-reinforced responses which were controlled by a schedule of reinforcement. Operant response rates were reduced after single overnight, irradiations with 2450 MHz (CW) microwaves at 10 or 15 mW/cm^2 when ambient air temperature during the exposure was 28°C but not when the temperature was 22°C. Decreases in response rate were accompanied by increases in response durations. Exposures to these ambient temperatures without microwave radiation produced no alterations in responding. Reduction in response rate was shown to be a function of exposure duration. Operant behavior of rats which was reduced by 50 percent or more after an overnight microwave exposure was not reduced after a similar exposure of only 55 min. In another experiment, response rates declined linearly with time for exposures lasting up to 4 hrs. No further decreases were seen for exposures lasting 4 to 8 hrs. Longer exposures again produced further rate decreases. Videotaped observations showed that rats did not change orientation or movement in relation to the electric or magnetic field vectors during irradiation with 2450-MHz microwaves with 15 mW/cm^2. However, mice showed increased movement but no preferred change in orientation when exposed to similar incident power levels. Orientation in the field would not lead to alterations in the amount of absorbed energy (specific absorption rate, SAR) in the rat but would in the mouse because of the relationship of body size to wavelength of the radiation. At 2450 MHz, energy absorption is greater when the mouse is oriented parallel to the electric field vector. Behavioral changes in laboratory rodents were seen to depend on ambient air temperature, duration of the exposure, and orientation in the field when such orientation alters energy absorption during microwave irradiation.

Key words: behavior, temperature, exposure duration, orientation, 2,450 MHz, mice, rats

DESCRIPTION OF METHODS

Several recent reviews indicate considerable experimental evidence of alteration in animal behavior by electromagnetic radiation (Justesen 1979, Gage 1984). Most experiments show that rates of performance, as measured by recording changes in spontaneously occurring activities or in a specific, learned behavior, are reduced by exposure at specific absorption rates (SARs) greater than 3 W/kg. The advantages of measuring specific, learned behavior as described in this report include high consistency and predictability of baseline performance.

Operant conditioning is frequently employed to train specific behaviors. In operant conditioning, a response (typically a depression of a lever) is followed by presentation of a reinforcer or reward. A pellet of food can serve as a reinforcer to a food deprived rodent. Presentation of a reinforcer increases the probability of occurrence of the behavior that preceeded it. In practice a reinforcer does not follow every response. The criterion for presentation of the reinforcer, or the schedule of reinforcement, determines the rate and temporal pattern of responses emitted by the subject. Typical schedules of reinforcement consist of presenting a reinforcer after a certain number of responses (called a fixed ratio schedule) or the first response after a specified time has elapsed since the last response (called a differential reinforcement of low rates) or last reinforcer (called a fixed interval schedule). In experiments described below, a nose poke into a food cup, as shown in Figure 1, was chosen as the operant response because it was easier to train than a lever press. Temporal patterns of responding are generally independent of the response used. Response rates and patterns of nose-poking are similar to those for lever pressing. Changes in operant behavior main-

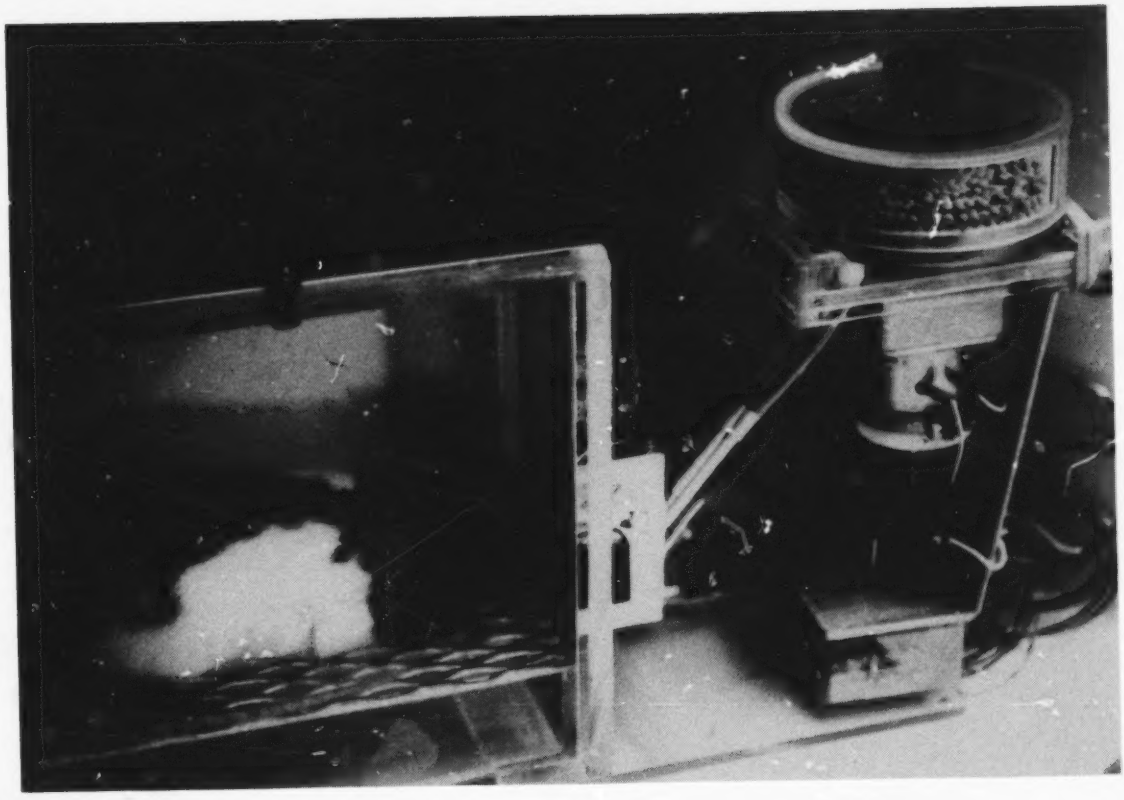

Figure. 1. Operant Conditioning Chamber for Nose-Poke Responses. Responses were detected by a photodiode detecting interruptions in a light path across the food cup at the lower right side of the testing box. Lights above the food cup signalled the schedule in effect to the rat. To the right of the testing box was a food dispenser. The testing apparatus was enclosed in a light and sound attenuating chamber.

tained by reinforcer schedules have been reported to occur both during and after exposures to electromagnetic radiation (Gage, 1984).

Results of several laboratory studies on rats and mice are described to illustrate the ways environmental factors may influence the magnitude of a toxicological effect. The effect was measured by a change in a specific behavior of an animal either during or subsequent to microwave exposure.

Electromagnetic radiation was limited to continuous waves (CW) at 2450 MHz because equipment to produce such exposures was readily available and 2450 MHz is a reference frequency used in most laboratory experiments. All irradiations took place in the far field of an anechoic chamber with the animals below a vertically directed rectangular horn antenna such that both the electric field (E-field) and the magnetic field (H-field) vectors were in the horizontal plane (Weil and Ali, 1984). To restrict orientation and movement in the field during exposures, the animals were placed in individual boxes made of acrylic or other plastic which was essentially transparent to the microwaves, . The restraining boxes were then placed inside the environmental chambers. One, or sometimes four, of the animals were exposed at the same time. During multiple animal exposures the boxes were positioned to optimize uniformity of incident power density.

The animals inside the restraining boxes were placed in an environmentally controlled plastic polyfoam chamber located within the anechoic chamber. Air temperature and humidity were set and maintained at desired levels in this environmental chamber. A second environmental chamber located adjacent to the anechoic chamber was used for simultaneous sham exposures. The air flowed in parallel between these two chambers so ambient air conditions were the same.

EFFECTS OF TEMPERATURE

Induction of thermal energy (heat) is a primary consequence of microwave irradiation. It is only too obvious that increases in air temperature could well augment microwave effects (Mumford,1969). However, endotherms have mechanisms to maintain body temperature in the face of environmental temperature changes. Moreover, the penetration and distribution of microwave energy within the body is different than it is for external radiant energy. Research by others has shown that microwaves alter behvioral thermoregulation of rats (Stern et al.,1979) and of squirrel monkeys (Adair and Adams,1980). Additionally, elevations in ambient temperature (Ta) can increase lethality due to microwaves (Rugh et al.,1975) or lead to a response which will result in a reduced rate of energy absorption (Monahan and Ho,1977).

Effects of air temperature on behavior that was not necessarily thermoregulatory but that was modified by microwaves were investigated in several experiments. The behavior chosen was response rate of rats on a random interval schedule of food reinforcement. This schedule was used to produce a medium rate of control response rates that were roughly linearly uniform during daily 30 min. sessions. After training, male, Long-Evans hooded rats were exposed three times at a Ta of 22°C, followed by three times at a Ta of 28°C, and one time again at a Ta of 22°C. Four rats each were exposed to incident power densities of 5 mW/cm^2, 10 mW/cm^2, and 15 mW/cm^2. The averaged whole body SAR for rats of similar size exposed in this anechoic chamber under far-field conditions is 0.2 W/kg for each mW/cm^2. Performance was measured after 15.5 hours of overnight exposure at different Ta's (Gage,1979b).

Response rate decreased only after exposures at the two higher power densities at a Ta of 28°C. Group response rates, expressed as mean of the percent of the control response rate for each rat (from the previous session) decreased after all three exposures at 10 or 15 mW/cm² at an Ta of 28°C as shown by the solid line in Figure 2. Changes in rate were not seen after similar exposures at 22°C, either before or after the exposures at 28°C. This effect was also seen in the data from individual rats.

The mean duration of a nose-poke was also measured for each rat. During sessions in which response rates decreased, response durations increased (Figure 3). Although the increases in response durations were highly variable, when considered ain conjunction with the decrease in response rate, they indicate an overall reduction in the activity of the rat. After exposures to other substances such as the drug scopolamine, decreases in response rates are not routinely accompanied by increases in response durations.

Response rates on a ratio schedule also decreased following microwave exposures. Eight male albino rats were trained to alternately press each of two response levers on the front panel of a testing box either 11 or 33 times to obtain a food pellet. These rats were exposed for 15 hours at power densities of 0, 5, and 10 mW/cm² at Ta's of 22, 25, 28, 31, and at 0 mW/cm² at 34°C.

Figure 4 shows that larger reduction in rates of responding were seen at lower Ta's when the exposures included microwaves than when they did not include microwaves. In both of the preceeding studies the degree of reduction in response rate was a function of the incident power density of the microwaves and the increase in Ta.

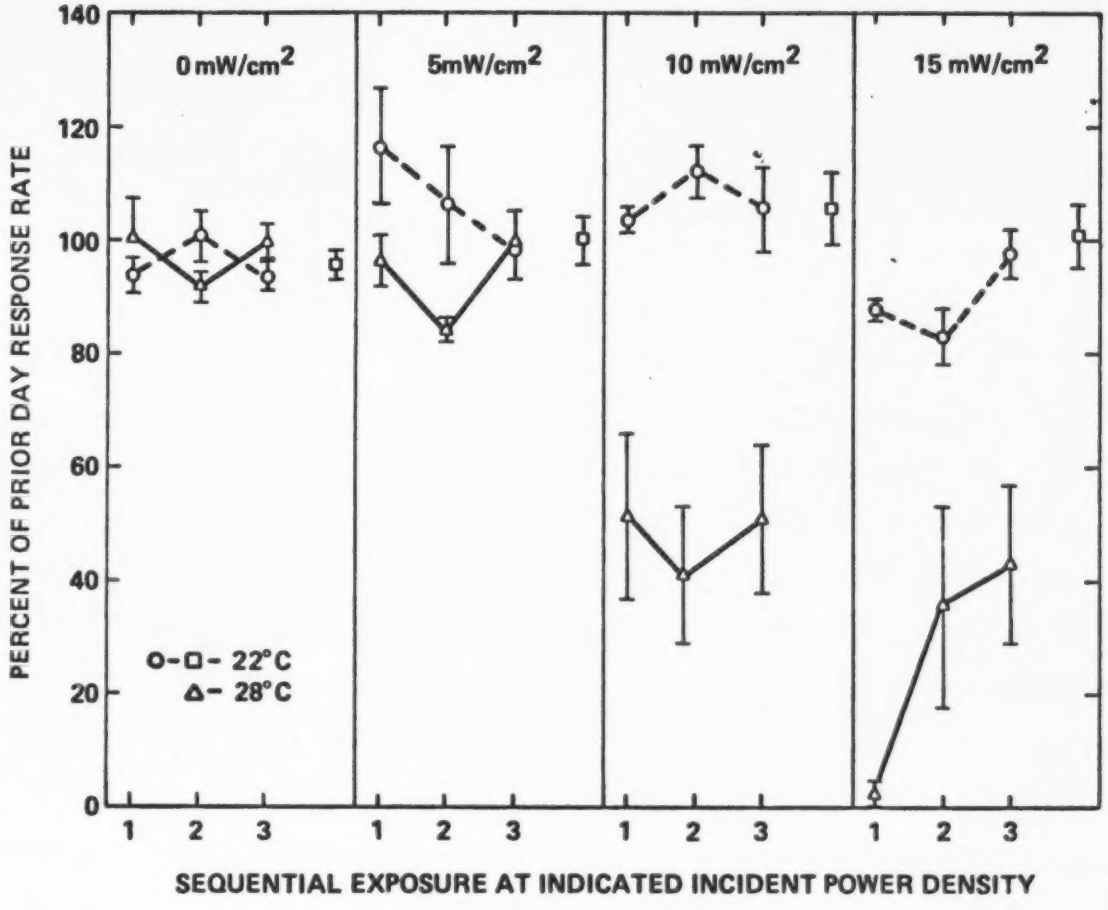

Figure 2. Interaction of Power Density and Ambient Temperature of Mean Response Rate. Points indicate mean ±SEM response rates for each group of 4 rats expressed as a percentage of response rate for the prior day of each rat. Points for 0 mW/cm^2 were obtained from data of all rats. The three points connected show data from three successive exposures at the indicated ambient temperature (reproduced from Gage, 1979b).

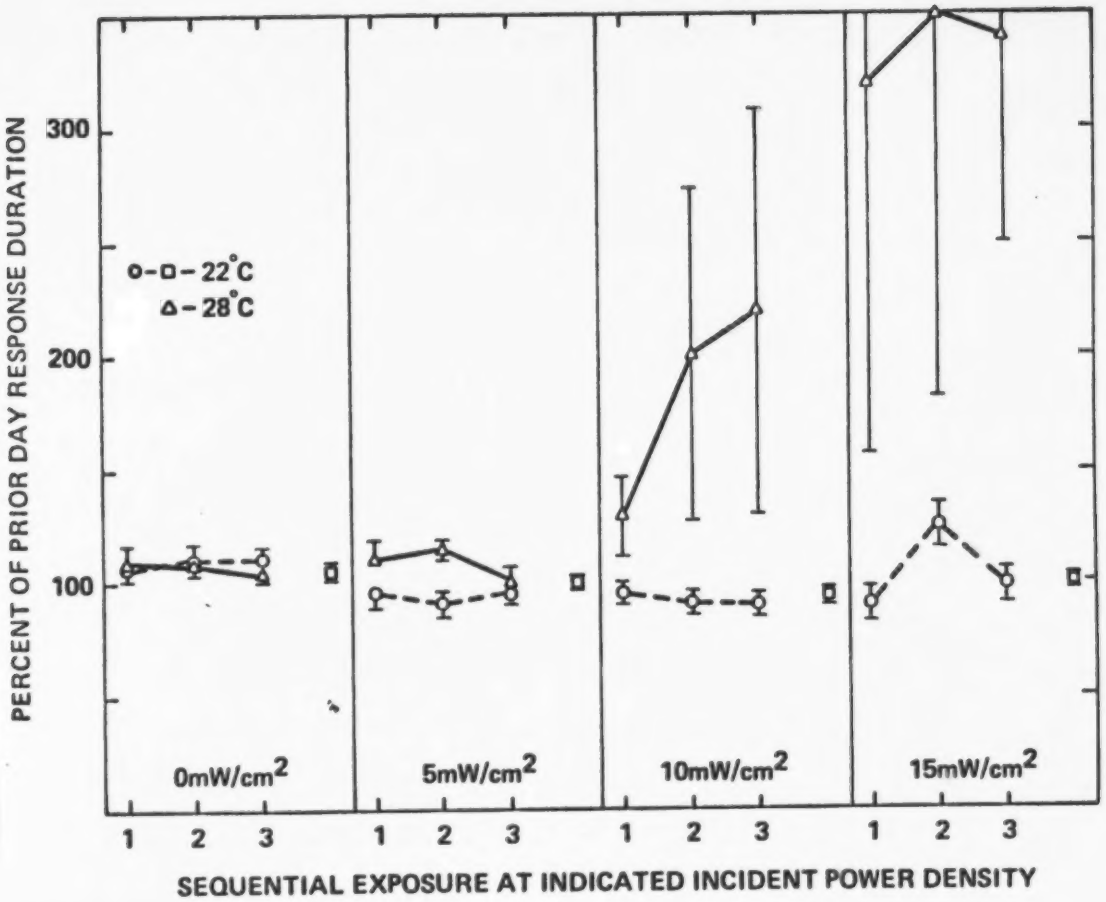

Figure 3. Interaction of Power Density and Ambient Temperature on Mean Response Duration. Points indicate mean ±SEM response durations for each group of 4 rats expressed as a percentage of mean response duration for the prior day of each rat. Points for 0 mW/cm^2 were obtained from data of all rats. The three points connected show data from three successive exposures at the indicated ambient temperature (reproduced from Gage, 1979b).

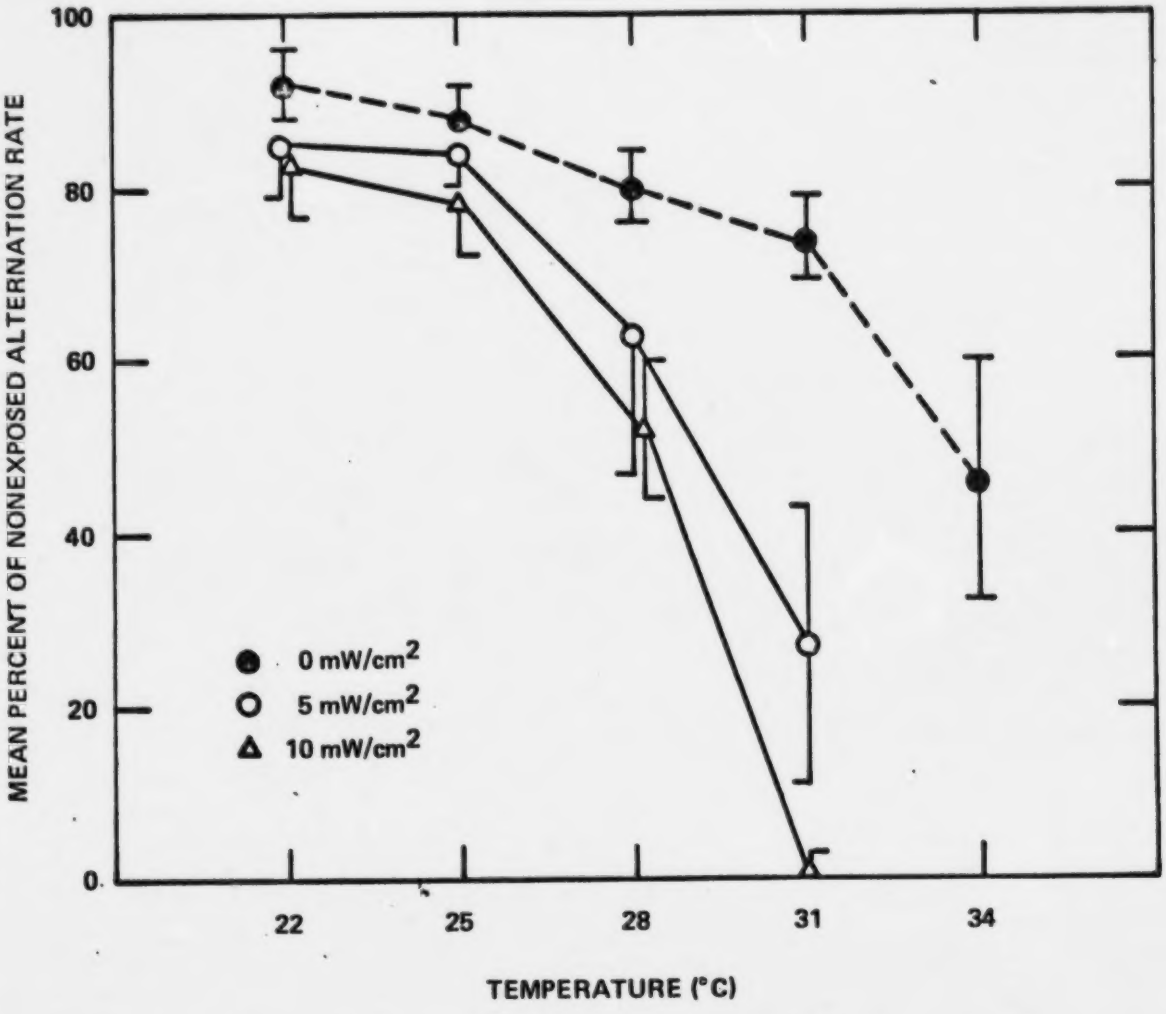

Figure 4. Interaction of Power Density and Ambient Temprature on Mean Alternation Rate. Mean (±SEM) of alternation rates of eight rats on a ratio of either 11 (for three rats) or 33 (for five rats) alternations between each of two levers for food pellet reinforcement expressed as a percentage of the rate in the prior test session for each rat. Each rat was exposed without microwaves (filled circles) and to 2450 MHz (CW) at 5 mW/cm^2 (open circles) and 10 mW/cm^2 (triangles) for 15 hrs at all air temperatures indicated by points. Animals were the same as those used in the experiment of Gage (1979a).

EFFECTS OF EXPOSURE DURATION

Behavioral effects of different exposure durations were assessed at a single ambient temperature, 22°C (Gage,1979). Animals were tested on the schedule requiring a fixed ratio of alternations between two levers for reinforcement after exposures lasting either 55 minutes or 15 hours. A repeated measures design was used such that each of eight rats was irradiated under all the conditions. These were the same animals used in the experiment described at the end of previous section, but the data were collected before those described in Figure 4.

Rates of alternating between the two levers decreased markedly after 15 hour irradiations with 10 mW/cm^2 or more, but decreased very little after 55 minute irradiations at power densities even as high as 30 mW/cm^2 (Figure 5).

In two subsequent studies effects of intermediate exposure durations were assessed by a variable interval 1 min (VI 1) schedule. A range of preprogrammed intervals, having a mean of 1 min, scheduled food pellet availablity with the next nose-poke response. This schedule produced stable linear response rates throughout and between each session. In the first experiment, 24 male albino rats were irradiated with 2450 MHz (CW) at 8 mW/cm^2 and 28°C for 0, 1, 2, 3, 8 and 16 hrs. The effect of exposure duration, as illustrated by the circles in Figure 6, was significant [$F(5,20) = 11.8$, $P < 0.0001$]. Duncan's multiple range test (at $\alpha = 0.05$) for post-hoc comparisons showed 0, 1, and 2 hr exposures were not different from each other, but were different from 4, 8, and 16 hr exposures. The 16 hr exposure was different from all the others. The shortest exposure duration that was significantly

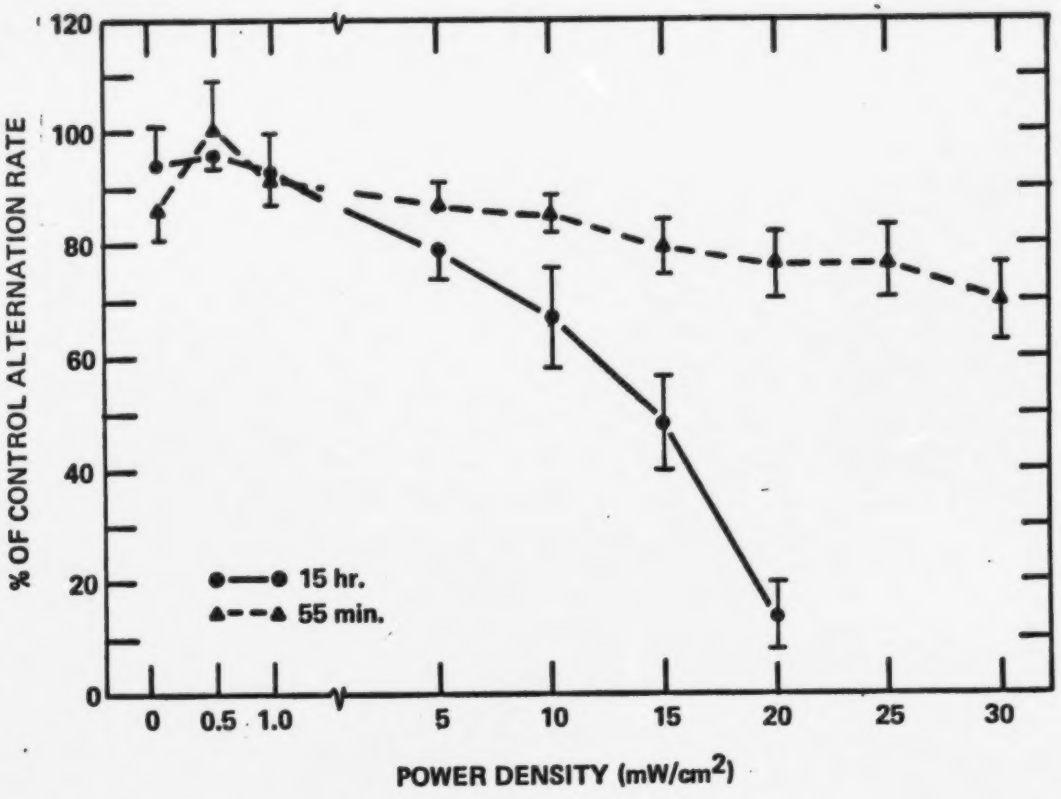

Figure 5. Interaction of Power Density and Duration of Mean Alternation Rate. Mean (±SEM) of response rates of eight rats required to alternate between each of two levers either 11 (for three rats) or 33 times) for five rats) for food pellets expressed as a percentage of the rate in the prior test session for each rat. Each rat was exposed to 2450 MHz (CW) for both 15 hrs (solid line) and 55 min (dashed line) at all power densities indicated by points (reproduced from Gage, 1979a).

different from control was 4 hrs. A linear regression analysis showed only 56 percent of the total variation of the response rates could be explained by a straight line. Therefore a curved line was hand fitted to the circle symbols in Figure 6.

These results on exposure duration were validated by a second experiment in which niave groups of 4 male hooded rats were exposed to 2450 MHz (CW) at 15 mW/cm^2 and 28°C for either 0, 4, 8, 12, or 16 hrs. When these data (represented by the squares in Figure 6) were connected by another hand fitted line, the nonlinear effect of decreasing response rate with increasing exposure duration was again seen. Data from additional groups of rats exposed for 0, 4, 12, or 16 hrs (represented by the triangles in Figure 6) further confirm the effect.

In both experiments, decreases in response rates were observed after exposures lasting as little as four hours. The percent decrease in response rate was the same for exposures lasting between four and 12 hours. The change in response rate increased further after exposures lasting over 12 hours. These results suggest rats may show compensation for effects during protracted exposure. However, the compensatory mechanism is no longer effective if the exposure persists beyond 12 hours. Compensatory mechanisms that produced similarly shaped three phase changes in body temperature with exposure duration were observed in earlier studies of dogs by Michaelson (1974). Long exposure durations may produce adaptive reponses mediated by the neuroendocrine system (Lu et al.,1977), but there is no information to directly link the behavior changes to any physiological effects.

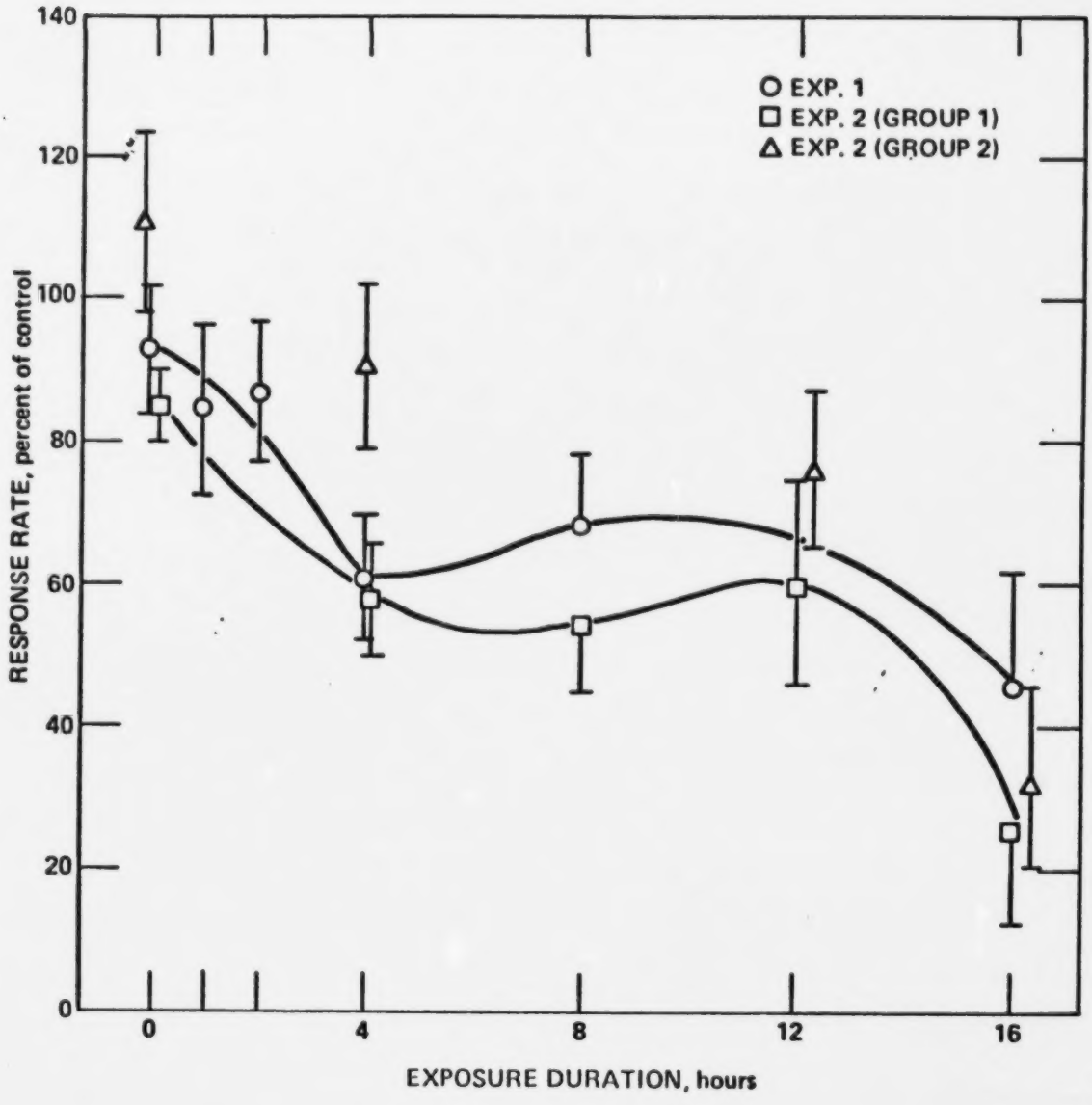

Figure 6. Effect of Exposure Duration on Mean Response Rate in Two Experiments. Points indicate mean ±SEM response rates for groups of rats expressed as a percentage of response rate for the prior day of each rat. All rats were tested on a VI 1 reinforcer schedule. Circles show data from 24 albino rats form experiment 1. Squares and triangles show data from groups of 4 hooded rats from experiment 2. Points from the same experiment were visually connnected by smooth lines.

EFFECTS OF ORIENTATION

Variations in the parameters discussed previously do not alter rate of energy absorption during exposure. By contrast, changes in orientation by an animal in a microwave field may result in alteration in the rate of energy absorption. The magnitude of this alteration depends on the size of the animal and the microwave frequency as indicated in the radiofrequency dosimetry handbook (Durney et al.,1980). At or near the resonant frequency for an animal, the organism will absorb more energy when oriented with the long axis of its body parallel to the electric field (E - field) vector than when oriented with the long axis of its body parallel to the magnetic field (H - field) vector. Differences in latency to onset of a tonic-clonic convulsion was reported to depend on body orientation at frequencies near body resonance in rats and mice (Schrot and Hawkins,1976).

To evaluate the effects of field orientation, videotapes were made of rats and mice exposed under a vertically directed horn antenna where they could move into positions parallel to the E, or H field vectors (Gage et al.,1979). The observation period lasted for 2 hours, with the microwaves turned on at 15 mW/cm^2 during the last hour. Animals were in a cuboidal, polyfoam chamber (or, during conditions not discussed here, in a cylindrical, acrylic chamber) at Ta's of 22 and 28°C. Animal positions in videotape samples taken every 30 seconds were scored for the long axis of the body parallel to the electric field vector (E), parallel to the magnetic field vector (H), or in another position (O) which was either curled or diagonally between both vectors.

Figure 7 shows that the rats did not orient preferably towards either the E - or H - field vectors. Moreover, they did not seem to change their behavior when the microwaves were turned on. Most often they were observed curled in a sleeping posture.

Activity of the mice increased when the microwaves were turned on. A greater number of time samples showed the long axis of the body of the mice oriented parallel to either the E - or H - field vectors. However, differences were not seen in in the amount of time spent oriented towards one vector as opposed to the other. This may have been because they did not have enough time to discriminate the behavior that would minimize the rate of energy absorption, although they detected some change in absorption occurred when they reoriented in the chamber. Levinson et al. (1982) reported that rats have difficulty learning specific responses that would lead to escape from lethal levels of microwaves unless another external stimulus was presented to indicate when the microwave intensity was decreased.

A comparison of the theoretical curves of SAR for rats and mice may explain why the mice showed a change in behavior while the rats did not. As shown in Figure 8 (modified from Durney et al.,1980), the resonant frequency for mice is near 2450 MHz and there are large differences in SAR depending on orientation of the animal with respect to the E - and H - field vectors at that frequency. The resonant frequency for rats is lower, and at 2450 MHz the SAR's with the body oriented parallel to either the E - or H - field vectors are similar. This theoretical relationship is reflected in actual measurements of the SAR's of rats and mice exposed in the polyfoam box under conditions described for this experiment(indicated by the symbols in Figure 8). The ratio of SAR for the two orientations (E/H) is 2.4/2.1 W/kg for rats,

which is approximately 1, but is 7.4/4.4 W/kg for mice, which is about 1.7. The higher SAR experienced by the mice may have been a sufficiently aversive stimulus to produce increased activity. However, the difference in SAR produced by orientation of the mice may not have been salient enough to direct this activity change towards lowering the SAR value.

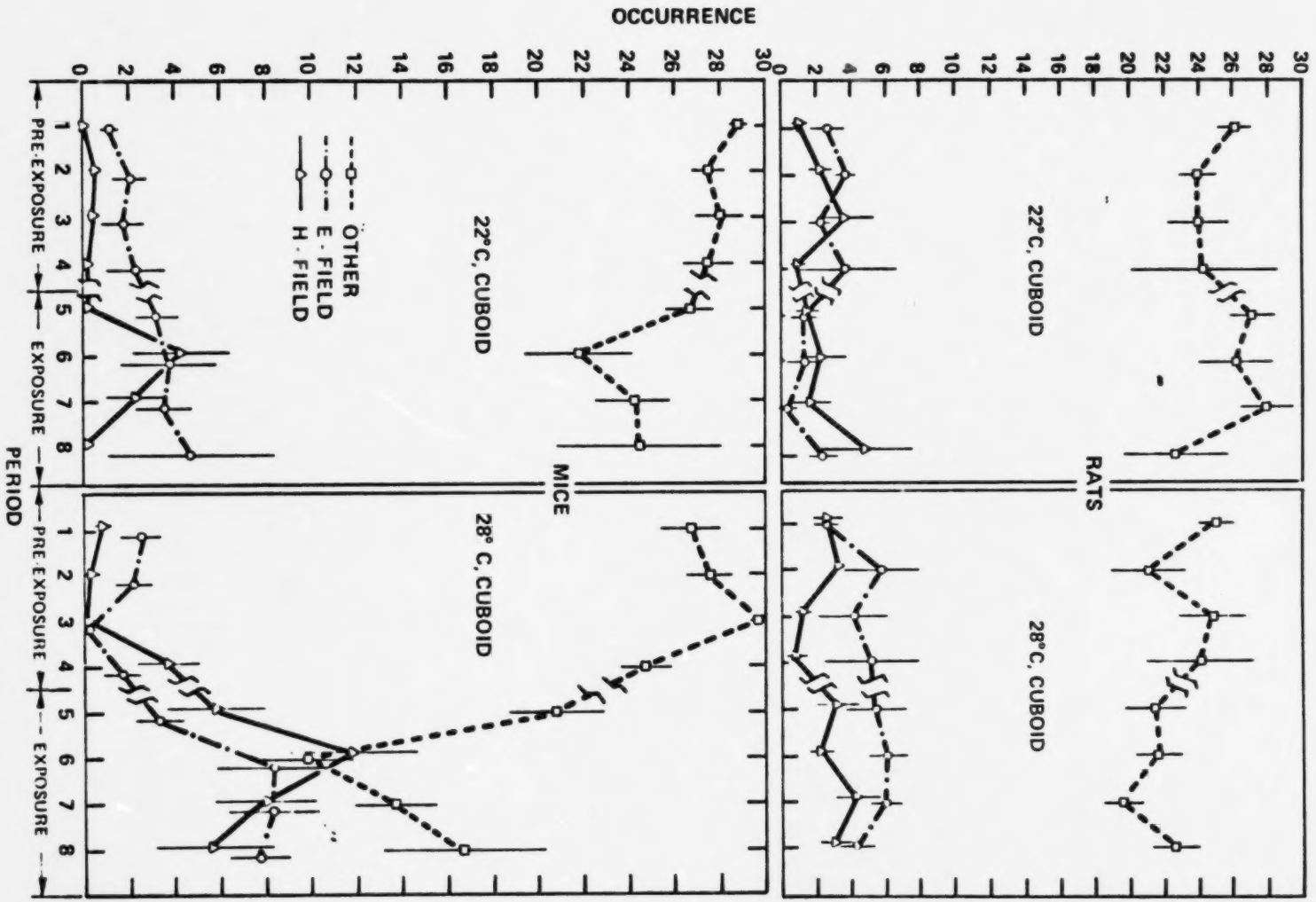

Figure 7. Effect of Exposure on Orientation of Rats and Mice at 22 or 28°C. Occurrences of position categories parallel to the electric field vestor (circles), parallel to the magnetic field vector (triangles), or in another orientation (squares) scored from videotapes for rats (upper panel) and mice (lower panel) for each 15 min period of a session. Each point indicates the mean (±SEM) of data from six animals that were observed in the cuboid foam plastic container at either 22 or 28°C. Thirty observations of each animal were made during every period. Exposure to 2450 MHz (CW) microwaves at 15 mW/cm^2 took place from periods 5 through 8 (adapted from Gage et al., 1979).

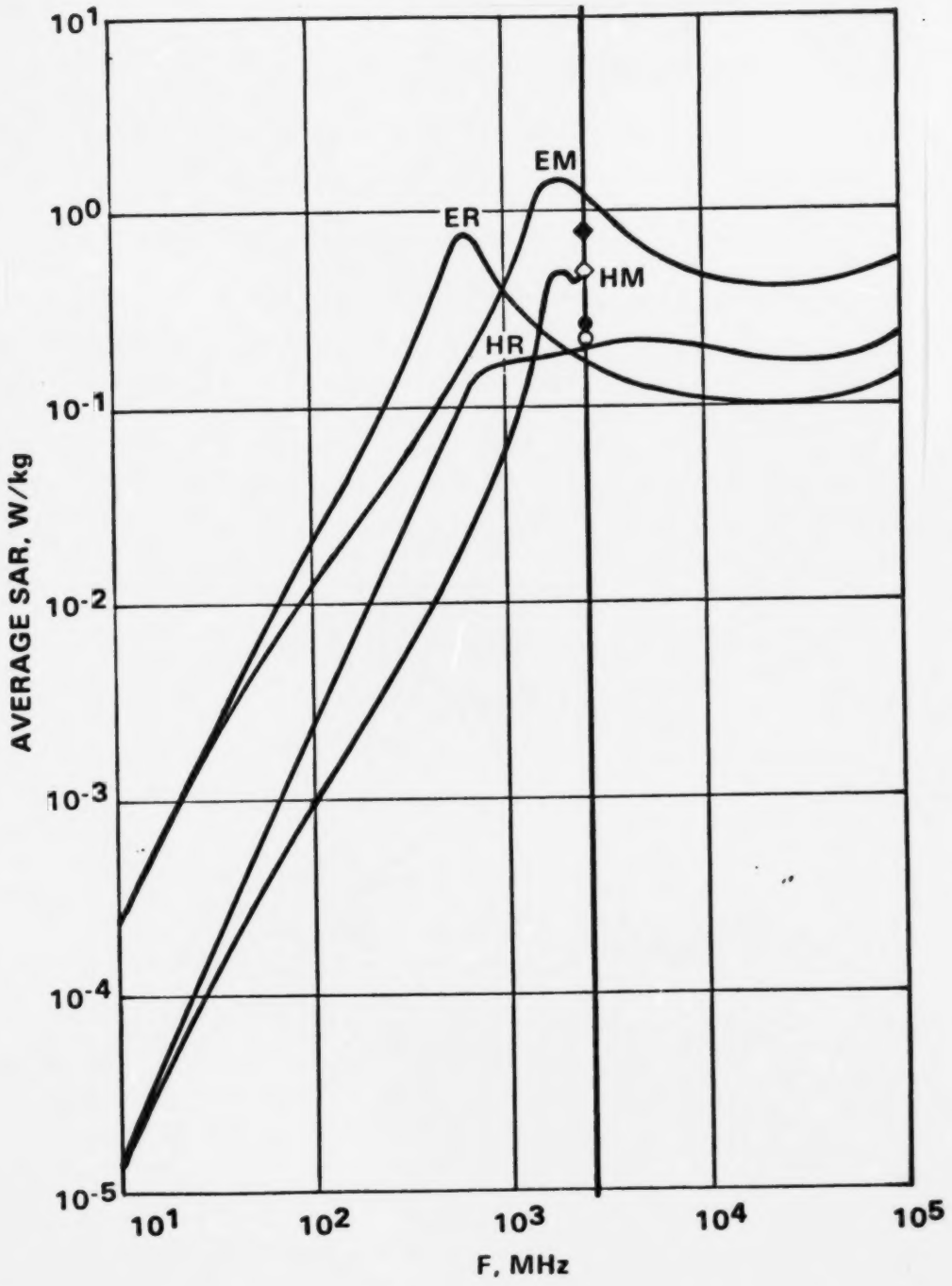

Figure 8. SAR of Rats and Mice Adapted from RF Dosimetry Handbook with Measured Vales Indicated. Curves show the calculated SAR for rats and mice oriented with the long axis of their body parallel to the electric field vector (ER and EM) or parallel to the magnetic field vector (HR and HM) of the microwave field as reproduced from the handbook (Durney et al., 1980). The circles show SAR determined for rats and the daimonds show SAR determined for mice by twin-well calorimetry under the conditions of exposure videotaped. Filled symbols indicate orientation with the long axis of the boby parallel to the electric field vector and open symbols indicate orientation parallel to the magnetic field vector.

CONCLUSIONS

Environmental conditions during exposure to electromagnetic radiation have been shown to interact with the parameters of the radiation to alter observed biological effects. Although these experiments were limited to measures of animal behavior, there is no reason to expect that such interactions may not be seen with other biological measures. Both the magnitude of behavior change and the treshold level needed to detect a change were modified by air temperature, duration of exposure, and orientation of the subject in relation to the propagation of the exposure. In real life situations, all these parameters may vary over the ranges explored in these experiments. Thought must be given to the extent to which laboratory findings made under a limited set of conditions, aside from those conditions intentionally varied, can be extrapolated to the conditions of daily exposure.

Only a small number of factors not directly altering the magnitude of the microwaves were investigated in these studies. There is no reason to believe that other factors may not also modify biological effects of electromagnetic radiation. The reviewed studies were conducted using a limited range of parameters of electromagnetic radiation. Many other experiments show effects of exposure may be highly dependent on the frequency, and the type of waveform as well as the power density. When environmental conditions are coupled with possible parameters of electromagnetic energy an innumerable set of exposure conditions may result which need exploration for determining the presence of toxicity. Possibly the best and only practical way to cope with this problem is to investigate exposure conditions that represent the extremes of each factor found in the environment. Unfortunately, too many toxicological

experiments choose environmental conditions that are historically based on the optimal conditions in basic biological research of the effect under study.

Ambient conditions may be an important parameter in specification of the toxicological threshold level for exposure to electromagnetic radiation and for specification of the exposure parameters that lead to toxicity of many other pollutants. Consideration should be given to the conditions of exposure in addition to the parameters of the toxicant itself in the setting of hazardous levels of exposure.

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